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Role of immunogen design in induction of soman-specific monoclonal antibodies☆

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Abstract

The study of monoclonal antibodies raised against defined hapten epitopes has been a useful approach to understanding antibody repertoire. The situation in which antibodies are raised against different epitopes of the same hapten but have some common recognition or binding features has been less frequently examined. To explore the latter situation, we have characterized three monoclonal antibodies previously raised against two structurally different epitopes of the same organophosphorus nerve agent hapten, pinacolymethyl phosphonofluoridate (soman). Two antibodies, BE2-IA10 (BE2) and CC1-IIA4 (CC1), raised against the hydrophobic pinacolyl motif of soman, bind exclusively to soman and not to any other organophosphorus nerve agents. We determined that these antibodies have the same heavy chain sequence, which they share with the unrelated antibodies MOPC 21 and H17-L19. While all these antibodies share the same heavy chain sequence, they each possess different light chain sequences. Binding studies revealed that each of these antibodies has a unique reactivity with a panel of structurally related ligands, suggesting that the light chains are critically important in determining specificity in these antibodies. The third antibody, #2.ID8.2, raised against the methyl phosphoryl portion of soman, has unique heavy and light chain sequences. This antibody binds to all the currently identified chemical warfare agents. Given that the presenting epitope used to induce #2.ID8.2 is common to sarin, soman, tabun and VX, the ability of this antibody to recognize each of these haptens versus the inability of BE2 or CC1 to do so demonstrates the important role that immunogen design can play in the specificity of an antibody response.

Keywords: Monoclonal antibodies; Soman; Nerve agents; Epitope

1. Introduction

The analysis of monoclonal antibodies raised against haptens has contributed to the understanding of antibody repertoire formation [1,2], somatic hypermutation [3–5] and affinity maturation [6]. When monoclonal antibodies raised in response to the same hapten are compared, they are often found to share similar heavy and light chain structures and fine specificities toward structurally related compounds [7,8]. Previously, several researchers have examined the antibody response to soman by using an immunogen in which a soman analog was coupled to a carrier protein through the phosphoryl moiety. Soman (Fig. 1a) is an organophosphorus chemical nerve agent with a fluorine-leaving group, and is an inhibitor of acetylcholinesterase capable of causing neurotoxicity in vivo [9,10]. Coupling of a soman-like moiety to a carrier protein was achieved by replacing the fluorine with a *p*-aminophenol group that was linked to the protein via a

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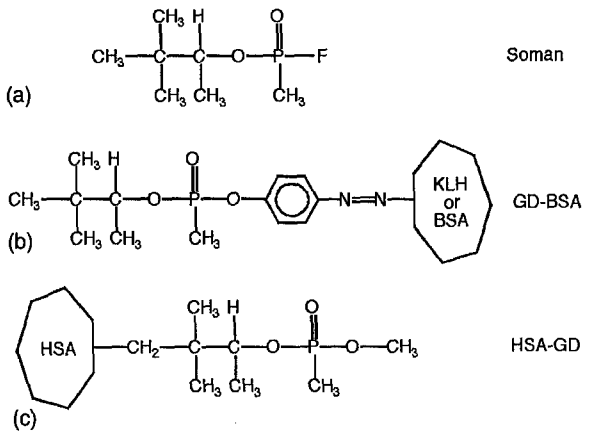


Fig. 1. Diagrams of soman (1a) and the two soman (1b and c) analogs used as immunogens to produce anti-soman antibodies.

diazo bond (Fig. 1b) [11–18]. This resulted in the aliphatic portion of the molecule being the most prominently presented portion of the epitope attached to the protein carrier. With this method, well characterized antibodies of moderate affinity were produced [12–18]. Within the panel of antibodies produced, variations in the variable region sequences were reported [17,18]. Previous studies reported that minor structural changes in the test haptens resulted in dramatic differences in antibody binding responses [12–14]. To further probe the structural elements of the hapten critical for epitope recognition, we examined the response of the immune system toward a soman analog in which the reactive organophosphorus end of the molecule extends away from the carrier protein, in an approach similar to that of Glikson et al. [19]. In the previous study, the fluorine of soman was replaced with either a hydrogen or hydroxyl group; here we have instead replaced the fluorine with an electronically similar methoxy group to ensure chemical stability in a physiologic environment (Fig. 1c). This soman analog was linked to a carrier protein through the pinacolyl group so that the presenting epitope was the phosphonate moiety (Fig. 1c). This hapten–carrier conjugate was used to immunize mice and produce B cell hybridomas. The antibody produced by one such hybridoma was compared with those of two hybridomas raised previously against the pinacolyl epitope of soman to determine how orientation of the hapten might affect the affinity, fine specificity and gene segment usage of responding antibodies. Each antibody displayed a unique specificity toward soman and other structurally related compounds. The two antibodies raised previously against the pinacolyl epitope of soman had distinct light chain gene usage, but shared identical heavy chains. Their affinities toward soman and related compounds differed, suggesting that the light chain structure was important in generating the specificity of these antibodies. The antibody raised against an analog of the reactive end of the soman molecule used unique heavy and light genes, and recognized four structurally distinct organophosphorus nerve agents.

2. Methods and materials

2.1. Hybridomas

The monoclonal antibodies CC1-IIA4 (CC1) and BE2-IA10 (BE2) were previously produced by hybridomas from mice that had been immunized with a *p*-aminophenol derivative of soman bound through a diazo linkage to either keyhole limpet hemocyanin (GD-KLH) or bovine serum albumin (GD-BSA) (for hybridoma CC1 and BE2, respectively) [12]. Hybridoma #2.ID8.2 was produced previously by Chanh and Lenz (unpublished results) from a female BALB/c mouse immunized with a soman-human serum albumin conjugate (HSA-GD) joined through the pinacolyl group (Fig. 1c). Hybridoma H17-L19 [20] as well as samples of A/PR/8/34 (PR8) and J1 influenza virus were kindly supplied by Walter Gerhard (Wistar Institute, Philadelphia, PA). The MOPC 21 antibody was obtained from Sigma–Aldrich (St. Louis, MO). Purified monoclonal antibody was obtained by passing tissue culture media through a protein G column followed by elution with 0.2 M gylcine, pH 2.7. Each fraction was neutralized in an appropriate volume of 1.0 M Tris, pH 9.0, dialyzed against phosphate buffered saline (PBS), and concentrated for subsequent use as previously described [12].

2.2. Enzyme linked immunosorbant assay (ELISA)

Both the purified monoclonal antibodies and unpurified cell culture supernatants were used in all immunoassays to determine binding, specificity and relative affinity of each antibody. ELISAs were performed as previously described [21]. Briefly, each antibody was placed in an Immulon II 96-well microtiter plate (Dynatech, Chantilly, VA) previously coated with GD-BSA, HSA-GD, HSA (Sigma–Aldrich), BSA (Sigma–Aldrich), PR8 or J1 in 0.1 M carbonate–bicarbonate coating buffer, pH 9.6. Plates were washed with PBS with 0.5% Tween 20 before the addition of goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma–Aldrich). After a 1 h incubation with the substrate *p*-nitrophenyl phosphate disodium (Sigma–Aldrich) dissolved in 10% (v/v) diethanolamine buffer, pH 9.8, the absorbance was read at 405 nm on a Molecular Devices (Sunnyvale, CA) microtiter plate reader.

2.3. Competitive inhibition enzyme immunoassay (CIEIA)

The CIEIA was performed as previously described [21]. Briefly, equal volumes of the antibody at a fixed concentration and competitor at varying concentrations were incubated together for 1 h before following the procedure previously described for ELISAs. CIEIAs were performed on all three antibodies against 17 different competitors. The nerve agents sarin (GB), soman (GD), tabun (GA) and VX were obtained from the US Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD. The

four stereoisomers of soman were obtained from the TNO Laboratory (Rijswijk, The Netherlands). Pinacolyl alcohol was purchased from Aldrich (Milwaukee, WI). Diisopropylmethylphosphonate (DIMP), dipinacolylmethylphosphonate (DPMP), hydrolyzed soman and hydrolyzed sarin were synthesized in-house from dichloromethylphosphonate and isopropyl or pinacolyl alcohol, respectively. All other inhibitors used in the CIEIA were purchased from Ash-Stevens Co. (Detroit, MI), and used as supplied. Based on the data from the CIEIA for each antibody/competitor combination, the concentration that inhibits 50% of antibody binding (IC_{50}) was determined [12].

2.4. Isotyping of antibodies

Two different assays for determining antibody light chain type and heavy chain isotype were performed on the supernatant of each hybridoma. The Isostrip™ Mouse Monoclonal (Boehringer Mannheim, Indianapolis, IN) antibody isotyping kit was used first on cell culture supernatants to determine the isotype as well as to assess whether antibody was being produced. The results were later confirmed by ELISA as described above, with the exception that the plates were coated with anti-isotype specific antibodies in 0.1 M carbonate–bicarbonate coating buffer, pH 9.6. These anti-isotype antibodies included anti-IgG_1, anti-IgG_{2A}, anti-IgG_{2B}, anti-IgG_3, anti-IgM, anti-kappa and anti-lambda (Sigma–Aldrich).

2.5. Sequence analysis of hybridoma H and L chain V-regions

Sequence analysis of hybridoma heavy (H) and light (L) chain variable (V) region gene segments was performed using primers complementary to conserved leader sequences and constant regions. Total cellular RNA was isolated from hybridomas (as previously described [22]) by washing 10^6 hybridoma cells with NTE (0.1 M NaCl, 10 mM Tris–HCl (pH 7.5, 1 mM EDTA)). The cells were lysed in NTE with 0.5% NP40 on ice for 5 min. Nuclei were pelleted by centrifugation for 5 min, and the supernatant was immediately transferred into digestion buffer (0.3 M NaCl, 0.2 M Tris–HCl pH 8.0, 25 mM EDTA, 2% SDS and 400 μg/ml of proteinase K). Samples were incubated for 1 h at 37 °C, and then extracted by vigorous vortexing with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). The phases were separated by centrifugation, and the aqueous phase was removed and retained. RNA was isolated from the aqueous phase by three rounds of ethanol precipitation. Complementary DNA (cDNA) was made using GibcoBRL Superscript Preamplification system (Gibco-BRL, Gaithersburg, MD). L-chain constant region specific primers used in the cDNA reaction were $C\kappa_2$ (5′-dGTTGGTGCAG-CATCAGC-3′) and $V\lambda_1$ (5′-dTCTCCTGGCTCTCAGCTCAG-3′), based on previously published work by Roark et al. [23]. Murine heavy chain constant region primers used in the reaction were Cγcross (5′-dGGGGCCAGTGGATAGAC-3′), Cμ (5′dAGACATTTGGGAAGGAC-3′) and Cγ3 (5′-dATGGGGCTGTTGTTTGTA-3′) [24]. The products of the reverse transcription reaction were amplified by the polymerase chain reaction (PCR) for 35 cycles using denaturation at 94 °C for 1 min, annealing at 50 °C for 2 min and extension at 72 °C for 2 min using primers described above as well as the following leader specific and/or V-region specific primers. The V_H regions were amplified with one of the following 5′-primers: V_H5'-1 (5′-dGAGGTGAAGCTGGTGGAG(A/T)-C(A/T)GG-3′) or V_H5'-2 (5′-dCAGGTCCAGTTGCAGCA-G(A/T)C(A/T)GG-3′) [24] as well as MHALT1.RV (5′-dGGGGATATCCACCATGG(A/G)ATG(C/G)AGCTG(T/G)GT(C/A)AT(C/G)CTCTT-3′), MHALT2.RV (5′-dGGGG-ATATCCACCATG(A/G)ACTTCGGG(T/C)TGAGCT(T/G)GGTTTT-3′) and MHALT3.RV (5′-dGGGGATATCCACC-ATGGCTGTCTTGGGGCTGCTCTTCT-3′) [25]. For amplification of V_L regions, L5 (5′-dCCAGATGAGCTCGTG-ATGACCCAGACTCCA-3′) [26], CK1 (5′-dAGATGGAT-ACAGTTGGT-3′) [24] and Cλ1 (5′-dCTTCAGAGGAAGG-TGGAAACAGGGTG-3′) [23] were used as well as ML-ALT1.RV (5′-dGGGGATATCCACCATGGAGACAGACA-CACTCCTGCTAT-3′), MLALT2.RV (5′-dGGGGATATCC-ACCATGGATTTTCAGGTGCAGATTTTCAG-3′), MLA-LT3.RV (5′-dGGGGATATCCACCATG(G/A)AGTCACA(G/T)AC(T/C)CAGGTCTT(T/C)(G/A)TA-3′), MLALT4.RV(5′-dGGGGATATCCACCATGAGG(G/T)CCCC(A/T)GCTCAG(C/T)T(C/T)CT(T/G)GG(G/A)-3′) and MLALT5.RV (5′-dGGGGATATCCACCATGAAGTTGCCTGTTAG-GCTGTTG-3′) [25]. PCR products were directly sequenced using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit version 2.0 (Applied Biosystems Inc., Foster City, CA) with both the 5′- and 3′-amplification primers, and run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc.). Sequences were analyzed using AutoAssembler 2.1 (Applied Biosystems Inc.) and SeqEd 1.03 (Applied Biosystems Inc.), and gene family assignments were made based on homology to previously sequenced antibodies as found in GenBank and Proteins of Immunological Interest [27]. The nucleotide sequences of both the heavy and light chains can be found in Genbank with accession numbers AY625275 (BE2 heavy chain), AY655276 (BE2 light chain), AY655277 (CCI light chain), AY655278 (CCI heavy chain), AY655279 (#2.ID8.2 light chain) and AY655280 (#2.ID8.2 light chain).

3. Results

3.1. Antibody isotype and gene sequences

All three antibodies were examined to determine their heavy chain isotype and the light chain type. The heavy and light chain variable region gene segments of #2.ID8.2 (IgG_{2A}-K), CC1 (IgG_1-K) and BE2 (IgG_1-K) were also nu-

Table 1
Antibody heavy chain CDR sequences

Antibody	Type	Family	H1	H2	H3
#2.ID8.2	IgG_{2A}	V_HQ52	GFSLISYGV	IWAGGRTN	CARDG...IREVYW J_H3
CC1	IgG_1	V_H7183	GFTFSSFGM	ISSGSSTL	CARWGNYPHYAMDYW J_H4
BE2	IgG_1	V_H7183	GFTFSSFGM	ISSGSSTL	CARWGNYPHYAMDYW J_H4
MOPC 21	IgG_1	V_H7183	GFTFSSFGM	ISSGSSTL	CARWGNYPYYAMDYW J_H4
H17L19	IgG_1	V_H7183	GFTFSSFGM	ISSGSSTL	CARWGNYPHYAMDYW J_H4

Each antibody was typed for the heavy chain by ELISA. The heavy chain nucleotide sequences were determined using a standard fluorescent dye terminator sequencing protocol; the deduced amino acid sequences of the heavy chain CDR regions are shown here, designated as H1, H2 and H3. The MOPC 21 sequence was from Hamlyn et al. [29] and Bothwell et al. [30] and the H17-L19 sequence came from Caton et al. [20]. The heavy chain CDR regions are designated as defined by Clothia and Lesk [31]. Gaps, indicated by dots, were introduced to facilitate sequence alignment.

cleotide sequenced (Tables 1 and 2). BE2 and CC1 have identical heavy chain sequences. After a database search with this sequence, we discovered that other antibodies, including MOPC 21 and H17-L19, also utilized this same heavy chain. These four antibodies, CC1, BE2, MOPC 21 and H17-L19, all have identical heavy chains with V_H7183 family V-region gene segments and JH4 J-region gene segments, yet all use unique light chains. The light chain of BE2 belongs to the V_K1 light chain gene family, that of CC1 belongs to the V_K9, while H17-L19 uses a V_K2 family gene and MOPC 21 uses a V_K19 light chain gene. There is only 46.8% sequence identity between the light chain variable regions of BE2 and CC1, which shows that these two light chains are not closely related. In contrast to these two anti-soman antibodies, #2.ID8.2 is unique in both the light chain (V_K24) and heavy chain (V_HQ52) gene segment usage (Tables 1 and 2).

3.2. Binding properties

All five antibodies (#2.ID8.2, BE2, CC1, H17-L19 and MOPC 21) were analyzed by ELISA using different capture antigens to determine the binding characteristics of each antibody. Since the antibodies raised against soman were induced in response to different carrier proteins and different soman analog haptens, each antibody was tested against GD-BSA, HSA-GD, HSA alone, BSA alone, PR8 (H17-L19 binds to the hemagglutinin of the PR8 virus) and J1 (a reassortant virus between PR8 and A/Hong Kong/1/68, in which the PR8 hemagglutinin has been replaced by a serologically non-cross reactive H3 hemagglutinin). Monoclonal antibody #2.ID8.2, previously raised against a soman analog attached to HSA through the pinacolyl group, bound equally well to either of the soman immunogens (GD-BSA and HSA-GD), bound 10,000-fold less to HSA alone, and did not bind to BSA alone, PR8 or J1. Monoclonal antibodies CC1 and BE2, each previously raised against the *p*-aminophenol derivative of soman, bound only to the GD-BSA and not to HSA-GD, BSA alone, HSA alone, PR8 or J1. The MOPC 21 and H17-L19 did not cross react with any of the soman immunogens, and H17-L19 bound to PR8 but not J1 influenza virus.

3.3. Competitive inhibition enzyme immunoassays

The three anti-soman antibodies were tested in a CIEIA with 17 different competitors, and the IC_{50} of each competitor was determined (Figs. 2 and 3). BE2 and CC1, which were raised against the pinacolyl end of the soman molecule (GD-BSA and GD-KLH, respectively), both bound only to soman and the soman stereoisomers, but to none of the other chemical warfare agents studied, with BE2 binding better than CC1. CC1 had a poorer relative affinity (>1 mM) toward soman, DIMP, pinacolyl alcohol and #84, and was also inhibited by *p*-nitrophenyl-GD (PNO_2) and #62. BE2 bound to a racemic mixture of soman, but showed only slight stereoisomer selectivity for the (C-P+) isomer. BE2 was not inhibited by any of the remaining structurally related competitors except for PNO_2 and DPMP. The third monoclonal antibody, #2.ID8.2, bound to VX, sarin, soman and tabun as well as to each of the stereoisomers of soman. However, neither #2.ID8.2 nor either of the two previous antibodies cross reacted appreciably with hydrolyzed soman or hydrolyzed sarin. #2.ID8.2, which was raised against HSA-GD, failed to bind to #2712, #62 and #84, all of which possess an aromatic ring structure.

Table 2
Antibody light chain CDR sequences

Antibody	Family	L1	L2	L3
#2.ID8.2	V_K24	SKSLLHSNGITY	QMS	NLELPYTFGGG J_K2
CC1	V_K9	ITCHASQGLSGN	HGT	YSQFPPTFGGG J_K1
BE2	V_K1A	SQSLINSNGNTY	KVS	STHVPYTFGGG J_K2
MOPC 21	V_K19	LTCKASENVVTY	GAS	GYSYPYTFGGG J_K2
H17-L19	V_K2	NQSLLDSDGETY	LVS	GTHFPFTFGSG J_K4

Each antibody was typed for the light chain by ELISA. The light chain nucleotide sequences were determined using a standard fluorescent dye terminator sequencing protocol; the deduced amino acid sequences of the light chain CDR regions are shown here, designated as L1, L2 and L3. The MOPC 21 sequence was from Hamlyn et al. [29] and Bothwell et al. [30] and H17-L19 sequence came from Caton et al. [20]. The three light chain CDR sequences are designated as defined by Clothia and Lesk [31].

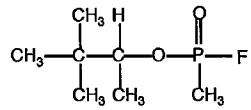
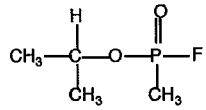
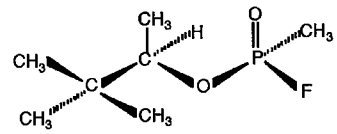
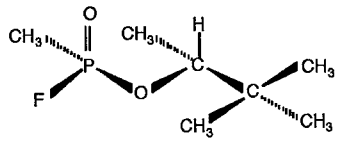
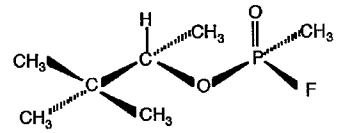
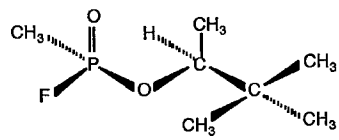
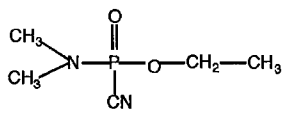
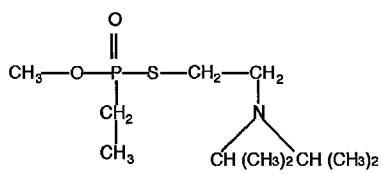
Inhibitors	Structures	CC1	#2-ID8.2	BE2
Soman (GD)		>1000	0.2	100
Sarin (GB)		N. I.	10	N. I.
Soman (C+P+)		>1000	0.09	>1000
Soman (C+P-)		>1000	1	500
Soman (C-P+)		>1000	1	200
Soman (C-P-)		>1000	18	>1000
Tabun (GA)		N. I.	13	N. I.
VX		N. I.	10	N. I.

Fig. 2. Names, structures and IC_{50} values (μM) of organophosphorous nerve agents used in the CIEIA. N.I.: no detectable inhibition. >1000: inhibition was detected, but did not reach 50% of uninhibited signal.

4. Discussion

Three monoclonal antibodies, #2.ID8.2, CC1 and BE2, were characterized as to their specificities, binding properties and gene usage. Two of these antibodies, CC1 and BE2, were raised previously against a hapten whose presenting epitope was the predominantly hydrophobic pinacolyl motif of the soman molecule. Upon probing the binding specificity of these two antibodies with a panel of 17 structurally related haptens, we found that they bind preferentially to racemic soman and, to a lesser extent, to the four stereoisomers of soman. In addition, BE2 also bound to DPMP, a symmetric soman analog, and to PNO_2, but not to any of the other haptens that contained aromatic groups. All of these compounds contain the same alkyl group that was present in the immunogen. Interestingly, BE2 was not inhibited by any of compounds that contained aromatic structures but lacked alkyl groups, even though the hapten against which it was raised did possess an aromatic structure. Likewise, BE2 did not recognize pinacolyl alcohol; together the data suggest that BE2 preferentially recognizes the pinacolyl portion of soman analogs when the phosphorus moiety is present. Using a slightly different array of test compounds, similar results were previously reported by Brimfield et al. [12]. In contrast, CC1 does exhibit some preference for both pinacolyl alcohol and the aromatic linker structure, as have many previously reported anti-soman antibodies [14,15,17]. Neither antibody binds to any of the other organophosphorus chemical warfare nerve agents tested (such as sarin, tabun or VX), demonstrating that these antibodies are highly specific for soman. The binding results for BE2 and CC1 are consistent with the pinacolyl (highly substituted aliphatic) end of soman

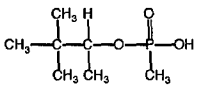
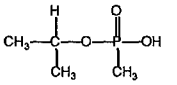
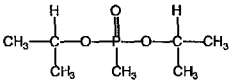
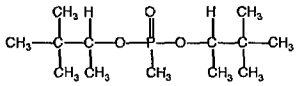
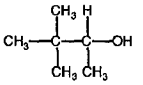
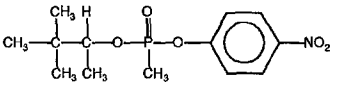
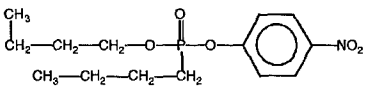
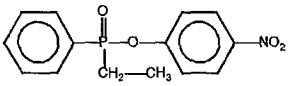
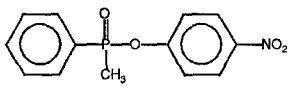
Inhibitors	Structures	CC1	#2-ID8.2	BE2
Hydrolyzed Soman		N. I.	N. I.	>1000
Hydrolysed Sarin		N. I.	N. I.	N. I.
Diisopropylmethylphosphonate (DIMP)		>1000	50	N. I.
Dipinacolylphosphonate (DPMP)		N. I.	4	2.5
Pinacolyl alcohol (Pin-OH)		1000	2.5	N. I.
p-Nitrophenylpinacolylmethylphosphonate (PNO_2)		10	5	400
p-Nitrophenyldibutylphosphonate (#2712)		N. I.	N. I.	N. I.
p-Nitrophenylethyl(phenyl)phosphinate (#84)		>1000	N. I.	N. I.
p-Nitrophenylmethyl(phenyl)phosphinate (#62)		7.5	N. I.	N. I.

Fig. 3. Names, structures and IC_{50} values (μM) of inhibitors used in the CIEIA. Compounds are also referred to by the name in parentheses due to simplicity. N.I.: no detectable inhibition. >1000: inhibition was detected, but did not reach 50% of uninhibited signal.

being the predominant binding determinant, and explain their specific binding to soman and not to the other agents. These findings are in good agreement with those reported by Brimfield et al. [12], who previously observed that the binding of similar monoclonal antibodies was highly sensitive to subtle structural changes in the aliphatic (pinacolyl) group.

The binding properties of #2.ID8.2, which was induced by a hapten that contained no aromatic group and was attached to the carrier protein in the opposite orientation relative to the BE2 and CC1 immunogen, did not mimic either of the two other anti-soman antibodies. In addition to binding to racemic soman and the four soman stereoisomers, #2.ID8.2 also bound to sarin, tabun and VX (Fig. 2). The inability of #2.ID8.2 to bind to any of the analogs containing aromatic groups except PNO_2 was not unexpected given that HSA-GD did not have any aromatic ring structures. It was unexpected that #2.ID8.2 was able to bind to pinacolyl alcohol and PNO_2, given that these structures have either no phosphonyl group or one that is linked to a bulky aromatic ring, respectively. The basis of this binding is currently being investigated, but for the latter structure, it may be that #2.ID8.2 is able to accommodate the bulky aromatic group in lieu of the smaller fluorine or methoxy groups, provided that the pinacolyl group is present as well. The inability of any of the three antibodies to bind to hydrolyzed soman can probably be accounted for by the fact that hydrolyzed soman (pinacolylmethyl phosphonic acid) is ionized in water and contains a full negative charge around the phosphonate motif. None of the other compounds in the test panel share that distinction; rather they share the pinacolyl motif absent any negative charge.

To examine the structural basis for the observed specificity differences among these antibodies, we determined the nucleotide sequence of their heavy and light chain variable regions. BE2 and CC1 were generated by fusion with the cell line P3X63, a derivative of MOPC 21 [28]. The deduced amino acid sequence of the BE2 and CC1 heavy chain variable regions are identical to those of MOPC 21 and P3X63, except for the substitution of a histidine for the third tyrosine in the CDR3 region (Table 1). This difference is a result of a single T to C nucleotide variation. The presence of this shared heavy chain rearrangement strongly suggests that the antibodies BE2 and CC1 (and also H17-L19) were not developed in vivo, but rather that the light chains of the original B cells paired with the heavy chain of the fusion partner during in vitro selection of the hybridomas. In all four antibodies, the V_L sequences differ markedly (Table 2), which presents a strong argument for the V_L chain playing a dominant role in hapten recognition. Our findings expand upon those of Buenafe et al. [17], who previously postulated that the V_L chains of anti-soman antibodies appeared to play a dominant role in generating their binding specificity. The observed difference in binding specificity for monoclonal antibody #2.ID8.2, i.e., its ability to bind to four organophosphorus nerve agents, including soman (both a racemic mix-

ture and the individual stereoisomers), sarin, tabun and VX (Fig. 2), suggested that a greater difference in V_L and V_H sequences may be responsible. This monoclonal antibody was raised toward the methyl-phosphonylmethoxide epitope, a mimic of the reactive end of the soman molecule where the fluorine-leaving group was replaced by the more stable methoxy group. #2.ID8.2 shared neither the fusion partner derived heavy chain gene found in BE2 or CC1 nor any detectable similarity in V_H or V_L sequence (Table 2). Given that the immunizing epitope for #2.ID8.2 was the more generic phosphonyl group, which is common to sarin, soman, tabun and VX, the ability of this antibody to recognize each of these haptens demonstrates the important role that immunogen design can play in the development of a hapten-specific antibody response.

These three antibodies raised against two different epitopes of the same hapten show unique fine specificities toward structural analogs of soman. The fact that simple changes in epitope presentation can result in substantial changes in the structures of induced antibodies suggests that considerable discrimination occurs during antibody selection. The results obtained here with BE2 and CC1 imply that binding specificity can be manipulated by making small changes in V_L chain nucleotide sequence without changing V_H sequence. This in turn suggests a simple process for making 'designer' anti-soman antibodies by introducing mutations into the light chains of BE2 and CC1. The fact that these three antibodies have different recognition specificity for soman and other organophosphorus chemical warfare agents raises the possibility that they could be used as immunodiagnostic reagents to distinguish between a generic exposure to organophosphorus chemical warfare agents or to a specific toxicant. In the future, we plan to identify the amino acid residues that contribute to the generation of soman specificity in these antibodies. With that information we plan to use site-directed mutagenesis to test the importance of the amino acids in the binding site as well as to explore the ability to make antibodies with altered fine specificities and substantially higher affinities for nerve agents.

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